ADVANCED ORGANIC CHEMISTRY

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ADVANCED ORGANIC CHEMISTRY

REACTIONS, MECHANISMS, AND STRUCTURE

FOURTH EDITION

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Adelphi University

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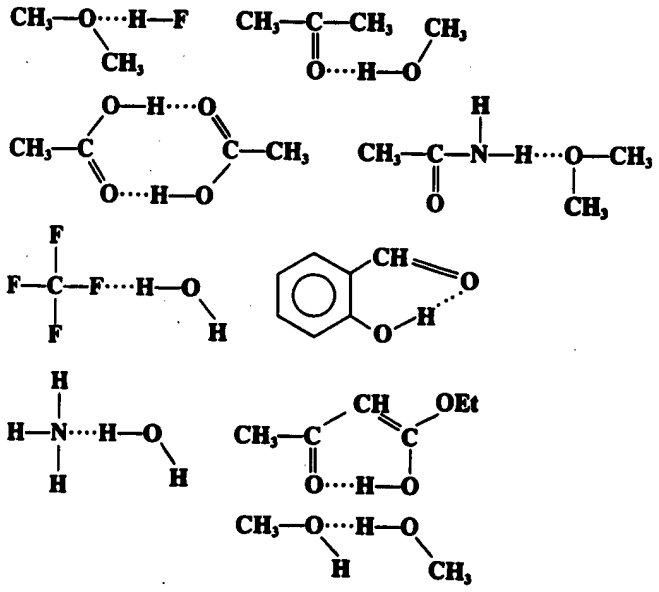
BONDING WEAKER THAN COVALENT

In the first two chapters we discussed the structure of molecules each of which is an aggregate of atoms in a distinct three-dimensional arrangement held together by bonds with energies on the order of 50 to 100 kcal/mol (200 to 400 kJ/mol). There are also very weak attractive forces *between* molecules, on the order of a few tenths of a kilocalorie per mole. These forces, called van der Waals forces, are caused by electrostatic attractions such as those between dipole and dipole, induced dipole and induced dipole, etc, and are responsible for liquefaction of gases at sufficiently low temperatures. The bonding discussed in this chapter has energies of the order of 2 to 10 kcal/mol (9 to 40 kJ/mol), intermediate between the two extremes, and produces clusters of molecules. We will also discuss compounds in which portions of molecules are held together without any attractive forces at all.

HYDROGEN BONDING

A *hydrogen bond* is a bond between a functional group A—H and an atom or group of atoms B in the same or a different molecule.[1] With exceptions to be noted later, hydrogen bonds are formed only when A is oxygen, nitrogen, or fluorine and when B is oxygen, nitrogen, or fluorine. The oxygen may be singly or doubly bonded and the nitrogen singly, doubly, or triply bonded. The bonds are usually represented by dotted lines, as shown in the following examples:



[1]For a treatise, see Schuster; Zundel; Sandorfy *The Hydrogen Bond*; 3 vols., North Holland Publishing Co.: Amsterdam, 1976. For a monograph, see Joesten; Schaad *Hydrogen Bonding*; Marcel Dekker: New York, 1974. For

Hydrogen bonds can exist in the solid and liquid phases and in solution. Even in the gas phase, compounds that form particularly strong hydrogen bonds may still be associated.[2] Acetic acid, for example, exists in the gas phase as a dimer, as shown above, except at very low pressures.[3] In solution and in the liquid phase, hydrogen bonds rapidly form and break. The mean lifetime of the $NH_3 \cdots H_2O$ bond is 2×10^{-12} sec.[4] Except for a few very strong hydrogen bonds,[5] such as the $FH \cdots F^-$ bond (which has an energy of about 50 kcal/mol or 210 kJ/mol), the strongest hydrogen bonds are the FH····F bond and the bonds connecting one carboxylic acid with another. The energies of these bonds are in the range of 6 to 8 kcal/mol or 25 to 30 kJ/mol (for carboxylic acids, this refers to the energy of each bond). Other OH····O and NH····N bonds have energies of 3 to 6 kcal/mol (12 to 25 kJ/mol). To a first approximation, the strength of hydrogen bonds increases with increasing acidity of A—H and basicity of B, but the parallel is far from exact.[6] A quantitative measure of the strengths of hydrogen bonds has been established, involving the use of an α scale to represent hydrogen-bond donor acidities and a β scale for hydrogen-bond acceptor basicities.[7] The use of the β scale, along with another parameter, ξ, allows hydrogen bond basicities to be related to proton transfer basicities (pK values).[8]

When two compounds whose molecules form hydrogen bonds with each other are both dissolved in water, the hydrogen bond between the two molecules is usually greatly weakened or completely removed,[9] because the molecules generally form hydrogen bonds with the water molecules rather than with each other, especially since the water molecules are present in such great numbers.

Many studies have been made of the geometry of hydrogen bonds,[10] and the evidence shows that in most (though not all) cases the hydrogen is on or near the straight line formed by A and B.[11] This is true both in the solid state (where x-ray crystallography and neutron diffraction have been used to determine structures),[12] and in solution.[13] It is significant that the vast majority of intramolecular hydrogen bonding occurs where *six-membered rings* (counting the hydrogen as one of the six) can be formed, in which linearity of the hydrogen bond is geometrically favorable, while five-membered rings, where linearity is usually not

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reviews, see Meot-Ner *Mol. Struct. Energ.* **1987,** *4*, 71-103; Deakyne *Mol. Struct. Energ.* **1987,** *4*, 105-141; Joesten *J. Chem. Educ.* **1982,** *59*, 362-366; Gur'yanova; Gol'dshtein; Perepelkova *Russ. Chem. Rev.* **1976,** *45*, 792-806; Pimentel; McClellan *Annu. Rev. Phys. Chem.* **1971,** *22*, 347-385; Kollman; Allen *Chem. Rev.* **1972,** *72*, 283-303; Huggins *Angew. Chem. Int. Ed. Engl.* **1971,** *10*, 147-151 [*Angew. Chem. 83*, 163-168]; Rochester, in Patai *The Chemistry of the Hydroxyl Group*, pt. 1; Wiley: New York, 1971, 327-392, pp. 328-369. See also Hamilton; Ibers *Hydrogen Bonding in Solids*; W.A. Benjamin: New York, 1968.

[2]For a review of energies of hydrogen bonds in the gas phase, see Curtiss; Blander *Chem. Rev.* **1988,** *88*, 827-841.

[3]For a review of hydrogen bonding in carboxylic acids and acid derivatives, see Hadži; Detoni, in Patai *The Chemistry of Acid Derivatives*, pt. 1; Wiley: New York, 1979, pp. 213-266.

[4]Emerson; Grunwald; Kaplan; Kromhout *J. Am. Chem. Soc.* **1960,** *82*, 6307.

[5]For a review of very strong hydrogen bonding, see Emsley *Chem. Soc. Rev.* **1980,** *9*, 91-124.

[6]For reviews of the relationship between hydrogen bond strength and acid–base properties, see Pogorelyi; Vishnyakova *Russ. Chem. Rev.* **1984,** *53*, 1154-1167; Epshtein *Russ. Chem. Rev.* **1979,** *48*, 854-867.

[7]For reviews, see Abraham; Doherty; Kamlet; Taft *Chem. Br.* **1986,** 551-554; Kamlet; Abboud; Taft *Prog. Phys. Org. Chem.* **1981,** *13*, 485-630. For a comprehensive table and α and β values, see Kamlet; Abboud; Abraham; Taft *J. Org. Chem.* **1983,** *48*, 2877. For a criticism of the β scale, see Laurence; Nicolet; Helbert *J. Chem. Soc., Perkin Trans. 2* **1986,** 1081. See also Nicolet; Laurence; Luçon *J. Chem. Soc., Perkin Trans. 2* **1987,** 483; Abboud; Roussel; Gentric; Sraidi; Lauransan; Guihéneuf; Kamlet; Taft *J. Org. Chem.* **1988,** *53*, 1545; Abraham; Grellier; Prior; Morris; Taylor *J. Chem. Soc., Perkin Trans. 2* **1990,** 521.

[8]Kamlet; Gal; Maria; Taft *J. Chem. Soc., Perkin Trans. 2* **1985,** 1583.

[9]Stahl; Jencks *J. Am. Chem. Soc.* **1986,** *108*, 4196.

[10]For reviews, see Etter *Acc. Chem. Res.* **1990,** *23*, 120-126; Taylor; Kennard *Acc. Chem. Res.* **1984,** *17*, 320-326.

[11]See Stewart *The Proton: Applications to Organic Chemistry*; Academic Press: New York, 1985, pp. 148-153.

[12]A statisical analysis of x-ray crystallographic data has shown that most hydrogen bonds in crystals are nonlinear by about 10 to 15°: Kroon; Kanters; van Duijneveldt-van de Rijdt; van Duijneveldt; Vliegenthart *J. Mol. Struct.* **1975,** *24*, 109. See also Ceccarelli; Jeffrey; Taylor *J. Mol. Struct.* **1981,** *70*, 255; Taylor; Kennard; Versichel *J. Am. Chem. Soc.* **1983,** *105*, 5761; **1984,** *106*, 244.

[13]For reviews of a different aspect of hydrogen bond geometry: the angle between A—H····B and the rest of the molecule, see Legon; Millen *Chem. Soc. Rev.* **1987,** *16*, 467-498, *Acc. Chem. Res.* **1987,** *20*, 39-46.

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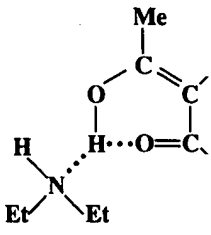

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Basic chemistry

Miklos Bodanszky

Peptide Chemistry

A Practical Textbook

With 2 Figures and 5 Tables

Springer-Verlag Berlin Heidelberg New York
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Professor Dr. Miklos Bodanszky
One Markham Road 1E
Princeton, NJ 08540, USA

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can take up only certain values. The allowable angles were calculated (Ramachandran et al. 1963) and are represented in "Ramachandran plots". These calculations and plots were vindicated in numerous studies of peptides and proteins by x-ray crystallography.

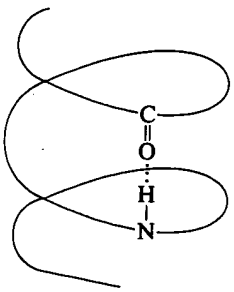
2. Secondary Structures

Polarization of the carbonyl group results in a partial positive charge at the oxygen atom, because it is more electronegative than the carbon atom. In the N–H bond of the amide group the nitrogen is the more electro-negative atom, hence a partial positive charge rests on the hydrogen. If the CO and NH groups are in sufficient proximity, a positive (attractive) interaction results, a "hydrogen bond" in which one of the unshared pairs of electrons of the oxygen atom provides a weak bonding effect

amounting to a few kilocalories per mole. In the hydrogen bond the hydrogen atom belongs, to some extent, to both electronegative atoms. In addition to oxygen and nitrogen, also atoms of sulfur and fluorine can serve as bridgehead. Hydrogen bonds are of extreme importance in the three dimensional structure of peptides and proteins. The cumulative effect of many weak attractive forces along the peptide backbone create well defined geometries and the forms stabilized by hydrogen bonds are called "secondary structure".

Early studies by Pauling, Corey and Branson (1951), involving x-ray structures of small peptides and experimentation with molecular models, resulted in the prediction of several geometric patterns in proteins. Of these the *α-helix* and *β-sheets* were subsequently encountered as dominant architectural features in numerous proteins. In more recent times also *reverse turns* (hairpin turns, γ-turns) were postulated (Venkatachalam 1968) and then recognized as frequent contributors to peptide and protein conformation.

The *α-helix* is a spiral structure stabilized by intramolecular hydrogen bonds between carbonyl oxygens and amide nitrogens four residues apart:



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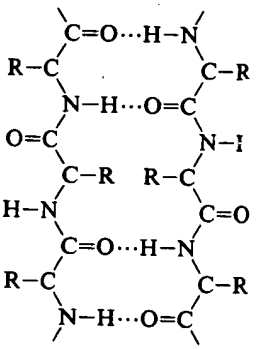

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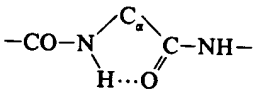
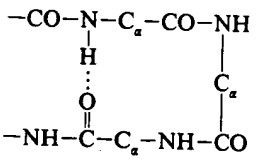


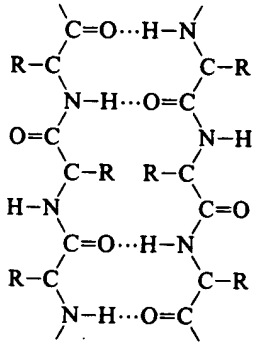
C_5

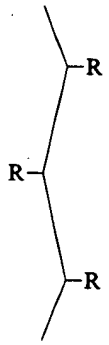


C_{11}

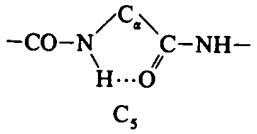
The hydrogen bonds are parallel to the axis of the helix and close a ring of 14 atoms including the hydrogen. There are thus 3.6 residues in one turn of the spiral which has a "pitch" (distance between turns) of 5.4 A. Accordingly a 5.4:3.6 = 1.5 A spacing was observed in x-ray diffraction patterns of powders of certain helical polyamino-acids and also of proteins of high helix content. This means that the same groups (CO, NH) repeatedly occur with a distance of 1.5 A between identical groups. It should be noted however, that there are several other, less frequently encountered helices, with different dimensions. For instance an elongated helix is present in polyproline and in the proline-rich protein collagen.

Peptide chains (or two parts of the same chain) connected with each other through multiple hydrogen bonds form *β-sheets*. The participating chains can be parallel or antiparallel to each other. An antiparallel sheet is shown here including a view along the general plane of the sheet to indicate its pleated character:





In *reverse turns* a sharp change in the direction of the peptide backbone is stabilized by an intramolecular hydrogen bond between a carbonyl oxygen atom and a nearby amide nitrogen. The ring thus formed can contain 5 or 8 or 11 etc. atoms and accordingly the conformation is designed as C_5 or C_8 or C_{11} etc.:



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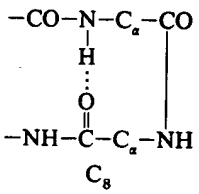

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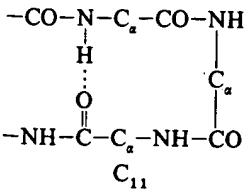


C_{11}

If one of the amino acid residues in a reverse turn has a configuration different from that of the other residues, then the hydrogen bond formed is more stable. In proteins and in the majority of biologically active peptides (which are usually cleavage products of proteins) only L-residues are present, but in microbial peptides D-residues are quite common. It is not surprising, therefore, that most of them have cyclic structures: ring closure is very much facilitated by reverse turns.

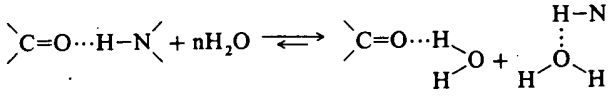
Stabilization of conformation by multiple hydrogen bonds is a *cooperative effect*. Disruption of a single hydrogen bond is not enough for destabilization, but the secondary structure collapses if a major part of the hydrogen bonds is disrupted. If compounds such as urea or guanidine salts, that are able to compete for the intramolecular hydrogen bonds are added to aqueous solutions of peptides or proteins the secondary structure disappears. The change does not take place gradually: at a certain urea etc. concentration a sudden collapse is noted: the structure "melts".

3. Tertiary Structure

Well defined folding of a peptide chain is called its tertiary structure. Hydrogen bond-stabilized reverse turns can contribute to folding but they are not its primary cause. A more important factor in folding was recognized in *non-polar interaction*, often described as *"hydrophobic bond"* (Kauzmann 1959).

When bulky side chains of aliphatic or aromatic amino acids approach each other a negative interaction (repulsion) would be expected between them. In aqueous solution, however, a positive interaction (attraction) is observed that can be explained with the escape of water molecules from the cage formed by non-polar regions. Inside such a cage water molecules are linked to each other by hydrogen bonds but obviously not to the surrounding non-polar amino acid side chains. These water molecules are, therefore, in a highly ordered state and order will decrease if they escape from the cage to join other water molecules outside. As the net effect of decrease in order (increase in entropy) the non-polar side chains are held close to each other by a "hydrophobic bond".

The importance of non-polar interaction can not be overemphasized. Hydrogen bonds which stabilize secondary structures should not exist in aqueous solutions of peptides and proteins. In the presence of a large number of water molecules the equilibrium



is expected to shift to the right and the hydrogen bonds between carbonyl carbons and amide nitrogens to be eliminated. Yet, non-polar interaction gen-

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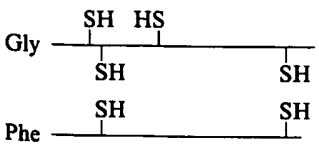

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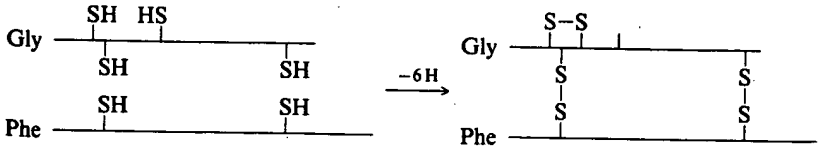
erates "hydrophobic pockets" in which there is no competition by water molecules for hydrogen bonds.

On one side of helices typically non-polar residues are found, with their side chains in contact with other hydrophobic residues further along the peptide chain. The attractive forces between such non-polar groups lead to a general folding of the chain, its "tertiary structure". The hydrophobic region created by the non-polar contacts within the fold contribute to the stability of the helix. Hence the expressions "secondary structure" and "tertiary structure" could be replaced by the term "secondary-tertiary structure". This interdependence between the two kind of structures extends to other hydrogen bond stabilized geometries, to β-sheets and reverse turns as well. Yet, stabilization of secondary structure by non-polar interaction can take place also without folding. Thus, hydrophobic bonds are found not solely between two sections of the same chain but also between two separate chains. Furthermore, intermolecular stabilization can be effected by molecules other than peptides. In numerous peptides helices are generated on addition of water-miscible alcohols, such as methanol, ethanol, isopropanol, tert.butanol, chloroethanol and particularly trifluoroethanol or hexafluoroisopropanol, to their aqueous solutions. Similar effects were observed on addition of detergents. These phenomena can be rationalized by the displacement of water from the proximity of amide groups and the ensuing elimination of competition for hydrogen bonds.

The role of disulfides in the determination of tertiary structure is less important than it would a priori appear. The architecture of peptides and proteins is primarily determined by the interaction of side chains of residues which are next neighbors and by long range cooperative effects of non-polar interactions. Disulfides form merely between sulfhydryl groups that are already in each others proximity. This was impressively demonstrated in the reduction and reoxidation of ribonuclease (White 1961). Instead of the formation of numerous disulfides different from those present in the parent molecule, the original disulfides were restored and the enzyme was regenerated with full biological activity. Also, random oxidation of six sulfhydryl groups in the two separated chains of insulin should produce a vast number of different molecules, but if prior to oxidation the two chains are first allowed to interact in aqueous solution then mainly the three disulfide bridges characteristic for insulin are formed



and insulin is the principal product.

A similar limited role can be assigned to *polar (coulombic) interaction.* Ion pair formation between basic and acidic side chains is quite commonly observed

in peptides and proteins. These "salt bridges", however, are not primary determinants of architecture. For instance, in secretin (conf. page 7) two ion pairs can be discerned, but in synthetic analogs with only one or no ion pair the original geometry of the molecule is not grossly altered. Thus, the contribution of disulfide bridges and ion pairs to the final determination of conformation in peptides consists mainly of providing certain rigidity to an already existing architecture.

4. Quaternary Structure

The expression "quaternary structure" is usually applied only for proteins. Their large molecules, containing hundred or more covalently bound amino acid residues, can assemble into still larger structures in which two or several "subunits" are linked through hydrogen bonds, polar or non-polar interactions. This kind of aggregation is known in myoglobin, hemoglobin and in numerous enzymes, all globular proteins. It appears, that at least some peptides are similarly prone to self-association. Thus insulin crystallizes in the form of a hexamer of the covalently built molecule while a trimer was revealed by x-ray crystallography in glucagon. (The primary structure of insulin and that of glucagon are shown on p. 7.) In both hormones self-association was accompanied by major conformational changes. In dilute aqueous solutions neither insulin nor glucagon exhibit pronounced helical character but molecules with high helix content are present in the crystals. The newly formed helical structures are the consequence of hydrophobic contacts between subunits and the ensuing displacement of water. Glucagon has a general tendency for self-association: under certain conditions β-structures appear in solution and aggregation can proceed to the point where insoluble fibrils separate. Fibril formation is known for insulin as well.

Self association seems to be related to a degree of conformational freedom. Smaller peptides with well defined architecture, such as oxytocin have not been observed in aggregated form so far. On the other hand many blocked intermediates in peptide synthesis exhibit annoying insolubility in organic solvents because of their tendency for aggregation in the form of β-sheets.

B. Methods for the Analysis of Conformation of Peptides

Electronic spectra yield valuable information on the presence or absence of chromophores and functional groups, but have rather limited use in the elucidation of the three dimensional structure in peptides. Infrared spectroscopy has been applied for the detection of helices and β-sheets, yet the spectra are usually meaningful only when the molecules are somewhat ordered as, for instance, in stretched films of polyamino acids. The scope of investigations seems to broad-

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1. Optical Rotatory Dispersion

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